Transnitrosation by *N*-Aryl-*N*-nitrosoureas; NO-Carrying *O*-Nitrosoisourea

Masayuki TANNO,* Shoko SUEYOSHI, and Naoki MIYATA

National Institute of Hygienic Sciences, 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158, Japan.
Received March 1, 1994; accepted April 18, 1994

Transfer of nitroso groups, so-called transnitrosation, from aromatic *N*-nitroso compounds such as *N*-nitrosoureas, *N*-nitrosamides and *N*-nitrosamines, to aromatic amines or ureas was observed under non-acidic conditions at room temperature. Sterically hindered 3,3-dibenzyl-1-(4-tolyl)-1-nitrosourea (1a) rapidly nitrosates indoline, *N*-alkylanilines or 3-methyl-1-(4-tolyl)urea to give their *N*-nitroso derivatives. In the case of *N*,*N*-dimethylanilines, nitrosative demethylation occurs to give *N*-methyl-*N*-nitrosanilines. The transnitrosation is accelerated by electron-releasing groups on the nitroso acceptors, *N*-alkylanilines. The transnitrosation mechanism is considered to be as follows: *N*-nitrosourea (1) thermally decomposes to nitric oxide and ureidyl radical followed by formation of an *O*-nitrosoisourea intermediate (10), which acts as an NO-carrying agent and nitrosates anilines or ureas.

Keywords nitrosourea; nitrosoisourea; nitrosamine, nitric oxide; dealkylation; transnitrosation

The migration of a nitroso group from *N*-nitroso compounds to nucleophilic species (secondary amines, alcohols, *etc.*), so-called transnitrosation, usually occurs under acidic conditions.[1,2] This reaction has been studied in connection with the production of carcinogenic nitrosamine in the human stomach.[3] Biological nitrosation by the catalytic action of bacteria has been observed even in high pH stomachs of patients with hypochlorhydria[4] and in the bladders of patients with urinary-tract infections.[5] Transnitrosation under non-acidic conditions[6] is little understood. We previously reported that *N*-aryl-*N*-nitrosoureas (**1**, **2**) generate nitric oxide (NO) *via* radical cleavage of an N–NO bond in a non-acidic solvent at room temperature. Generation of NO was confirmed by trapping as an *N*,*N*′-ethylenebis(salicylideniminato)iron–NO_3 complex.[7] The thermal decompositions of aromatic *N*-nitrosoureas (**1**, **2**) and *N*-nitrosamide (**3**) proceed by parallel reactions of radical cleavage of an N–NO bond (path A in Chart 1) and diazo ester rearrangement (path B) to give products such as ureas (**5**, **6**), nitro compounds (**14**), triazenes (**15**) and diazonium salts, as shown in Chart 1.[8–11]

Examples of thermal transnitrosation under non-acidic conditions through the radical N–NO bond cleavage pathway have been demonstrated only in aromatic *N*-nitrosamines,[6d] though no detailed study on transnitrosation has been conducted using acyl-type aromatic *N*-nitrosoureas and *N*-nitrosamides. This may be due to the ease of characteristic diazo ester rearrangement[12] rather than N–NO bond radical cleavage, in contrast to aromatic *N*-nitrosamines.

3-Benzyl-1-(4-tolyl)-1-nitrosourea (**2a**) and 3-methyl-1-(4-tolyl)-1-nitrosourea (**2b**) rearranged to the corresponding 3-nitroso isomers (**4a**,**b**) by intramolecular 1,3-nitroso shift from the N^1-position toward the N^3-position of the ureido group in non-acidic solvents at 33 °C.[9,10] Intermolecular nitrosation of 3-methyl-1-(4-tolyl)urea (**6b**) with **2a** as well as 3-isopropyl-1-(4-tolyl)-1-nitrosourea (**2c**) gave 3-methyl-1-(4-tolyl)-3-nitrosourea (**4b**) in carbon tetrachloride at 33 °C.[9,10]

The present paper describes non-acidic transnitrosation using various *N*-nitroso compounds (**1**—**4**, **7**, **8**), as shown in Fig. 1. The relationship between ease of transnitrosation and structural characteristics of *N*-nitroso compounds is discussed.

Results and Discussion

Transnitrosation with 3,3-Dibenzyl-1-(4-tolyl)-1-nitrosourea (1a) to *N*-Alkylanilines (9) Compound **1a** reacted with *N*-alkylanilines (**9**) in CCl_4 to produce *N*-alkyl-*N*-nitrosanilines (**8**) accompanied with 3,3-dibenzyl-1-(4-tolyl)urea (**5a**). The yields of transnitrosation products (**8**) were 12—93% (Chart 2, Table I). The time-course of *N*-nitrosaniline (**8**) formation in the reactions of **1a** and *p*-substituted *N*-methylanilines (**9a**—**e**) is shown in Fig. 2. The formation of the denitrosated product, **5a** increased

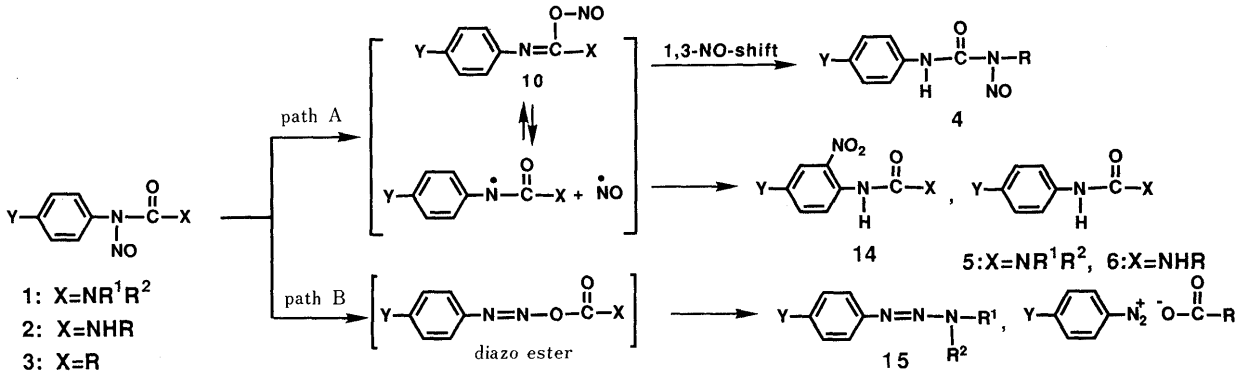

Chart 1

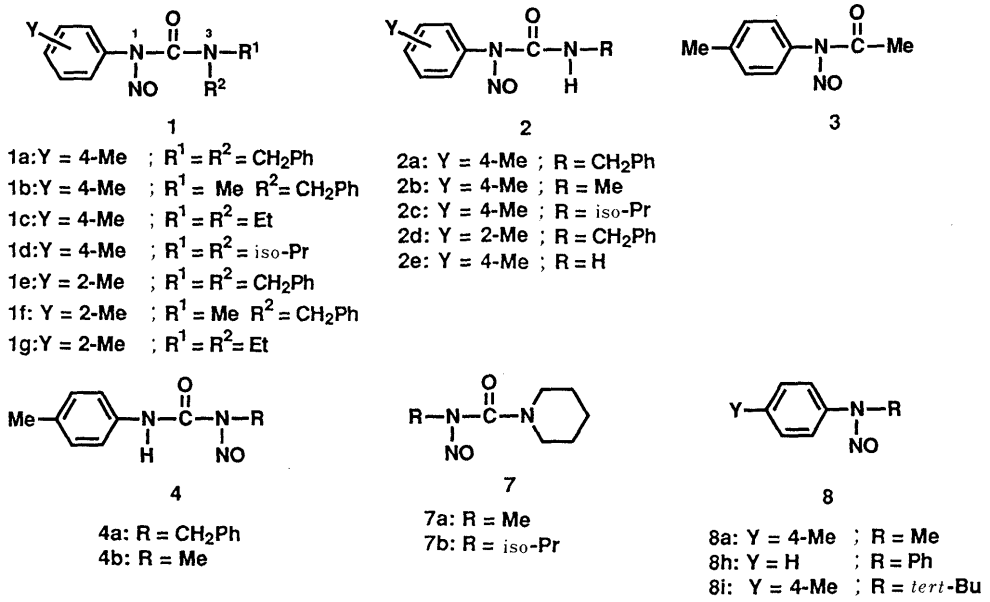

Fig. 1

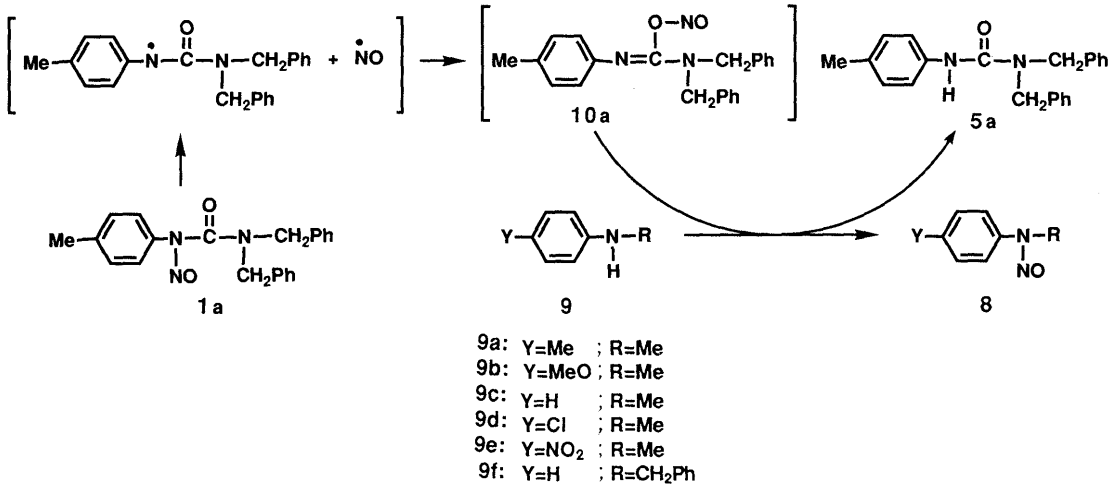

Chart 2

TABLE I. Yields of *N*-Nitrosamines (**8**) Formed in the Reaction of *N*-Alkylanilines (**9**) with 3,3-Dibenzyl-1-(4-tolyl)-1-nitrosourea (**1a**) in CCl_4 at 20 °C[a]

Compd.	Y-C_6H_4NHR		Reaction time (h)	Yield[b] of **8** (%)
	Y	R		
9a	4-Me	Me	5.5	53
9b	4-MeO	Me	3.0	93
9c	H	Me	5.5	42
			1.2[c]	53
9d	4-Cl	Me	5.5	42
9e	4-NO_2	Me	5.5	12
			0.8[c]	67
9g	H	Bn	1.1[c]	49

a) Concentrations of **1a** and **9** were 4.20×10^{-3} M. Bn = benzyl. *b*) Determined by HPLC. *c*) Reacted at 33 °C.

TABLE II. Rate Constant for the Decomposition of 3,3-Dibenzyl-1-(4-tolyl)-1-nitrosourea (**1a**) in the Presence of *N*-Methylanilines[a] (**9**) in CCl_4 at 20 °C

Compd.	Y-C_6H_4NHMe Y	Concentration of **1a** $\times 10^{-3}$ M	Rate constant[b] $k \times 10^{-4}$ s^{-1}
9a	4-Me	1.0	0.92
9b	4-MeO	1.0	3.18
9c	H	1.0	—
9d	4-Cl	0.25	0.98
		1.0	0.92
		2.0	1.12
		4.0	1.12
9e	4-NO_2	1.0	0.92
		1.0	0.55[c]

a) Concentration of *N*-methylanilines (**9**) was 1.0×10^{-3} M. *b*) Rate constant for the disappearance of **1a**. *c*) Rate constant for the decomposition of **1a** in the absence of **9**.

with the production of *N*-nitrosanilines (**8**). In this transnitrosation, electronic effects of *p*-substituents of *N*-methylanilines (**9a—e**) were observed: electron-releasing groups at the *p*-position of anilines increased the yield of **8**, while electron-attracting groups decreased them, implying that the basicity of aniline nitrogen of **9** influences

the reaction yield.

In the reaction of **1a** and *N*-methyl-4-chloraniline (**9d**), the decomposition rate for **1a** obeyed first-order kinetics (Table II), though formally, it should have followed second-order kinetics. First-order kinetics was also noted in the transnitrosation of **1a** to 3-methyl-1-(4-tolyl)urea (**6b**). The rate constants ranged from $0.92 \times 10^{-4}\,s^{-1}$ to $1.12 \times 10^{-4}\,s^{-1}$. Nevertheless, there was variation in the ratio of **1a** and **9**, and the rate constant was close to the value ($0.55 \times 10^{-4}\,s^{-1}$) in the self-decomposition of *N*-nitrosourea (**1a**). Thus, an intermediate appears to participate in the reaction. We previously proposed an *O*-nitroso intermediate (**10**)[7,10] in the self-decomposition of acyl-type aromatic *N*-nitroso compounds (Chart 1). Such an intermediate (**10**) may be a nitrosating agent in transnitrosation. Our proposed non-acidic transnitrosation mechanism is illustrated in Chart 2. The N–NO bond radical cleavage of *N*-nitrosourea (**1a**) occurs first by thermal decomposition. The resulting NO reacts with ureidyl radical to produce the *O*-nitroso intermediate (**10a**). This intermediate acts as an NO-carrying agent and leads to the urea (**5a**) after nitrosating **9**.

The disappearance rate constant of *N*-methyl-4-methoxyaniline (**9b**) was exceptionally large among *p*-substituted anilines, possibly due to the powerful electron-releasing effect of the MeO group and particularly high

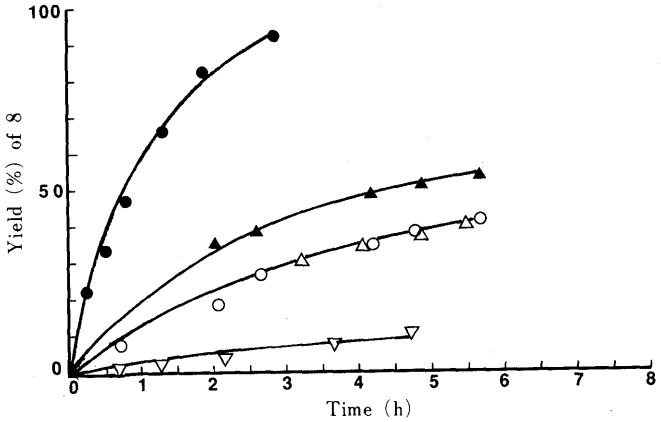

Fig. 2. Formation of *N*-Nitrosanilines (**8a—e**) by the Reaction of *p*-Substituted *N*-Methylanilines (**9a—e**) with 3,3-Dibenzyl-1-(4-tolyl)-1-nitrosourea (**1a**) in CCl_4 at 20 °C

●, *p*-OMe; ▲, *p*-Me; △, *p*-H; ○, *p*-Cl; ▽, *p*-NO_2.

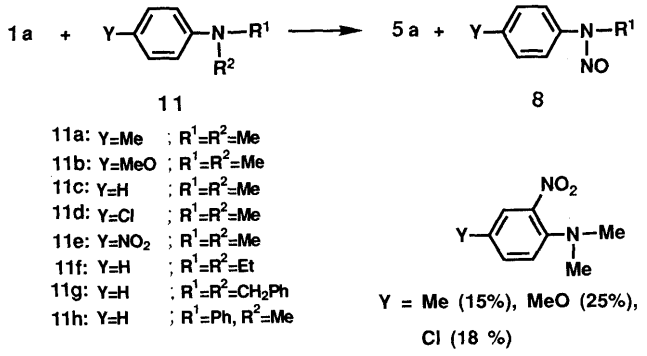

Chart 3

TABLE III. Yields of *N*-Nitrosamines (**8**) Formed in the Reaction of Tertiary Amines (**11**) with 3,3-Dibenzyl-1-(4-tolyl)-1-nitrosourea (**1a**) in CCl_4 at 20 °C[a]

Compd.	Y-$C_6H_4NR^1R^2$			Reaction time (h)	Yield[b] of **8** (%)
	Y	R^1	R^2		
11a	4-Me	Me	Me	0.83	54
11b	4-MeO	Me	Me	1.3	25
11c	H	Me	Me	1.3	25
				0.9[c]	47
				24.0[d]	47[e]
11d	4-Cl	Me	Me	2.0	25
11e	4-NO_2	Me	Me	6.0	—
				6.0[c]	—
11f	H	Et	Et	0.78[c]	46
11g	H	Bn	Bn	0.87[c]	12
11h	H	Ph	Me	0.57[c]	6

a) Concentrations of **1a** and **11** were 4.20×10^{-3} M. Bn = benzyl. *b*) Yield by HPLC. *c*) Reacted at room temperature. *e*) Isolated yield.

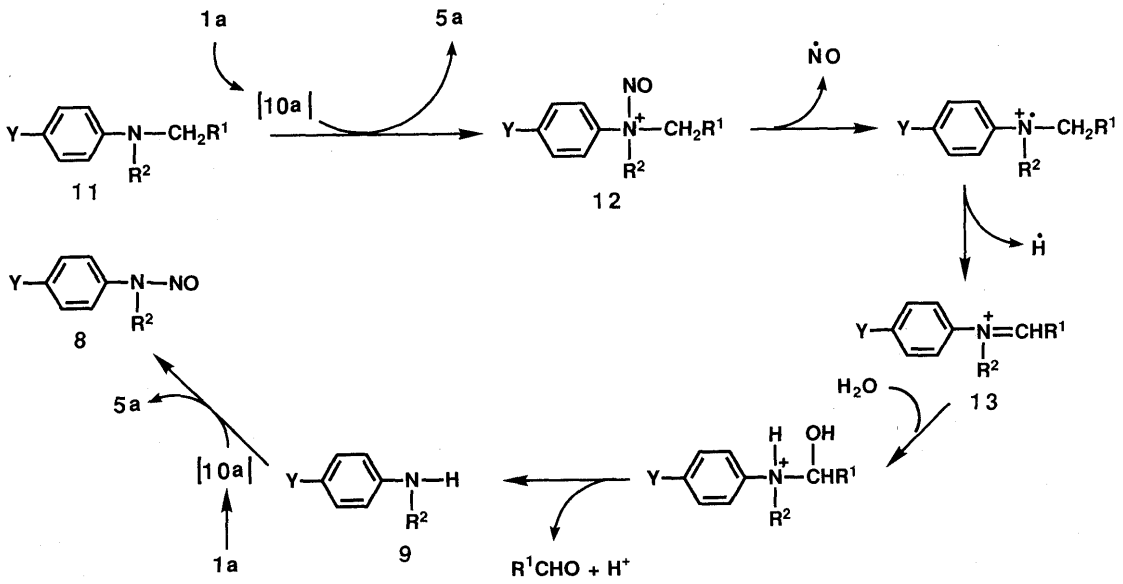

Chart 4

reactivity of **9b** toward the intermediate (**10**). A clear explanation for the abnormal rate constant is not available at present.

Dealkylative Nitrosation of *N,N*-Dialkylanilines (11) with 1a Under similar reaction conditions to those used for *N*-alkylanilines (**9**), **1a** reacted with *N,N*-dimethylanilines (**11a—e**) to give the demethylated nitrosative products, *N*-methyl-*N*-nitrosanilines (**8a—e**) in 6—54% yields (Chart 3, Table III). They were identified by nitrosation of the corresponding *N*-methylanilines (**9**). Formaldehyde was detected in the reaction of **1a** with *N,N*-dimethylaniline (**11c**). The reaction of **1a** with *N,N*-diethylaniline (**11f**) and *N,N*-dibenzylaniline (**11g**) gave *N*-ethyl-*N*-nitrosaniline (**8j**) and *N*-benzyl-*N*-nitrosaniline (**8k**), respectively. In the case of *N*-methyldiphenylamine (**11h**), *N*-nitrosodiphenylamine (**8h**) was obtained by demethylating nitrosation. Nitrosative dearylation was not observed. The formation of *N,N*-dimethyl-2-nitraniline derivatives in 15—25% yields together with the corresponding dealkylated nitrosation products (**8a, b, d**) was observed.

The proposed mechanism for demethylating transnitrosation is shown in Chart 4. After the *O*-nitroso intermediate (**10a**) derived from the N–NO bond radical cleavage of *N*-nitrosourea (**1a**) reacts with *N,N*-dialkylaniline (**11**) to produce a nitrosammonium ion (**12**), **12** decomposes to give iminium ions (**13**). The addition of water to the ions (**13**) gives aldehyde and *N*-monoalkylaniline (**9**), and the resulting **9** is nitrosated again with **1a** to form *N*-alkyl-*N*-nitrosaniline (**8**). This non-acidic dealkylating nitrosation mechanism is similar to the Leoppky–Tomasik mechanism[13a] for dealkylating nitrosation under acidic conditions using sodium nitrite in glacial acetic acid at 85—90 °C, and to the Verardo–Giumanini–Strazzolini mechanism[13b] for dealkylating nitrosation by refluxing of *N,N*-dimethylanilines with excess alkyl nitrites under non-acidic conditions. Our non-acidic dealkylation occurred at room temperature. This difference is due to homolytic fission, which proceeds preferentially in aprotic solvents, and *N,N*-dialkylanilines are considered to be predominantly nitrosated with the *O*-nitroso intermediate rather than the nitrosonium cation (NO^+) intermediate.

The formation of a nitro compound appears to support NO radical release from **12**.[10]

Transnitrosation with *N*-Nitrosoureas (1) to 3-Methyl-1-(4-tolyl)urea (6b) Compound **6b** was used as a nitroso group acceptor having a less basic amido nitrogen instead of anilines. When three *N*-nitrosoureas, 3,3-dibenzyl- (**1a**), 3-benzyl-3-methyl- (**1b**), and 3,3-diethyl-1-(4-tolyl)-1-nitrosourea (**1c**), were used as transnitrosating agents, **6b** was converted to 3-methyl-1-(4-tolyl)-1-nitrosourea (**2b**) and its 3-nitroso isomer (**4b**) (Chart 5) in the yields given in Table IV.

Formation curves of transnitrosated products (**2b, 4b**) are compared in Fig. 3a and 3b. Compound **1**, having bulky substituents, rapidly gave **2b** and **4b**. The first-order rate constants decreased in the order of bulkiness of the N^3-alkyl groups; **1a** (dibenzyl) > **1b** (benzyl,methyl) > **1c** (diethyl). It is of particular interest that 1-nitrosourea (**2b**) was produced predominantly compared with the 3-nitroso isomer (**4b**). The basicity of the N^1-nitrogen of the urea (**6b**) adjacent to the tolyl group must surely have been less that of the N^3-nitrogen bonded to the methyl group. The formation of **2b** started earlier in the reaction than that of **4b**. Time-courses of formation of the products obtained by reaction of **1c** with **6b** indicated a 1,3-shift of the nitroso group after 6 h.

Transnitrosation with *N*-Nitroso Compounds to Indoline (16) When *N*-methylanilines (**9**) or indoline (**16**) of high basicity were used as nitroso acceptors in the transnitrosation of **1c**, the self-decomposition product, 3,3-diethyl-1-(4-tolyl)triazene (**15c**), was not observed. *N*-Methyl-*N*-nitrosaniline (**8c**) or *N*-nitrosoindoline (**17**) was produced

TABLE IV. Rate Constant for the Decomposition of 3,3-Dialkyl-1-(4-tolyl)-1-nitrosoureas (**1a—c**) and Yields of 3-Methyl-1-(4-tolyl)-1-nitrosourea (**2b**) and Its 3-Nitroso Isomer (**4b**) in the Reaction of **1a—c** with 3-Methyl-1-(4-tolyl)urea (**6b**)[a] in CCl_4 at 33 °C

Compd.	Y-C_6H_4N(NO)CONR^{1}R^2			Rate constant[b] ($k \times 10^{-4}$ s^{-1})	Reaction time (h)	Yield of products (%)[c]	
	Y	R^1	R^2			**2b**	**4b**
1a	4-Me	Bn	Bn	3.49	2.0	17.0	7.4
				2.94[d]	2.0	—	—
1b	4-Me	Bn	Me	1.35	2.0	10.4	2.0
					4.0	17.5	3.7
				1.12[d]	2.0	—	—
1c	4-Me	Et	Et	0.65	2.0	2.5	0.0
					4.0	4.2	0.0
					6.0	8.0	16.0
				0.60[d]	2.0	—	—

a) Initial concentrations of *N*-nitrosoureas (**1a—c**) and 3-methyl-1-(4-tolyl)urea (**6b**), 3.4×10^{-3} M. *b*) Rate constant of the decomposition for the *N*-nitrosoureas (**1a—c**). *c*) Determined by HPLC. *d*) Blank, self-decomposition of *N*-nitrosourea (**1**).

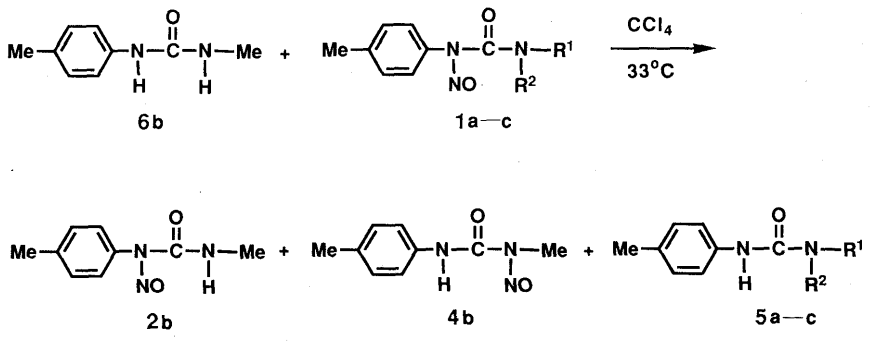

Chart 5

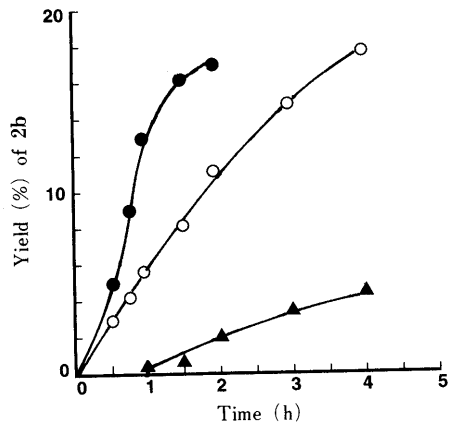

Fig. 3a. Formation of 3-Methyl-1-(4-tolyl)-1-nitrosourea (**2b**) by the Reaction of 3,3-Dialkyl-1-(4-tolyl)-1-nitrosourea (**1a—c**) with 3-Methyl-1-(4-tolyl)-1-nitrosourea (**6b**) in CCl_4 at 33 °C

●, **1a**; ○, **1b**; ▲, **1c**.

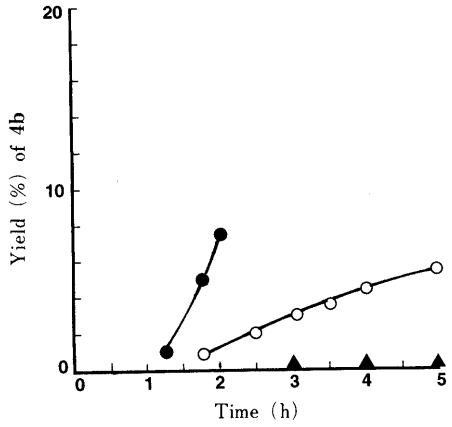

Fig. 3b. Formation of 3-Methyl-1-(4-tolyl)-3-nitrosourea (**4b**) by the Reaction of 3,3-Dialkyl-1-(4-tolyl)-1-nitrosourea (**1a—c**) with 3-Methyl-1-(4-tolyl)urea (**6b**) in CCl_4 at 33 °C

●, **1a**; ○, **1b**; ▲, **1c**.

TABLE V. Yields of *N*-Nitrosoindoline (**17**) Formed in the Reaction of Indoline (**16**) with *N*-Nitroso Compound (**1—3, 7, 8**) in CCl_4 at 33 °C[a]

Compd.	X-N(NO)-Y		Reaction time (h)	Yield[b] of **17** (%)
	X	Y		
1a	4-Tol	$CON(Bn)_2$	0.5	88
			10.0	87[c]
1b	4-Tol	CONMeBn	1.2	75
1c	4-Tol	$CON(iso\text{-}Pr)_2$	1.0	62
1d	4-Tol	$CON(Et)_2$	1.2	47
1e	2-Tol	$CON(Bn)_2$	1.0	73
1f	2-Tol	CONMeBn	1.7	80
1g	2-Tol	$CON(Et)_2$	2.2	90
2a	4-Tol	CONHBn	8.0	59
2d	2-Tol	CONHBn	24.0	7
2e	4-Tol	$CONH_2$	3.0	26[d]
3	4-Tol	COMe	0.8	43
7a	Me	$CONC_5H_{10}$	24.0	0
7b	iso-Pr	$CONC_5H_{10}$	24.0	0
8a	4-Tol	Me	24.0	16
8h	Ph	Ph	24.0	53
8i	4-Tol	*tert*-Bu	24.0	9

a) Concentrations of *N*-nitroso compounds and indoline were 4.20×10^{-3} M. Bn = benzyl, Tol = tolyl. *b*) Determined by HPLC. *c*) Reacted at 0 °C. *d*) Reacted in $CHCl_3$.

in fairly good yield. Many aromatic *N*-nitroso compounds (**1—3, 8**) reacted with indoline (**16**) to give *N*-nitorosoindoline (**17**) in carbon tetrachloride at 33 °C. The results of transnitrosation are summarized in Table V. The yield of **17** per unit time was excellent when trisubstituted *N*-aryl-*N*-nitrosoureas (**1a—g**) were used. With 3,3-dibenzyl-1-(4-tolyl)-1-nitrosourea (**1a**), a particularly high yield of 88% was obtained in 0.5 h. Among disubstituted *N*-aryl-*N*-nitrosoureas (**2a—d**), the 4-tolyl derivative (**2a**) gave **17** in moderate yield, but the 2-tolyl isomer (**2d**) formed **17** in only 7% yield after 24 h. With aliphatic *N*-nitrosoureas (**7**), no *N*-nitrosoindoline (**17**) was produced.

N-Nitroso-4-tolylacetamide (**3**) produced *N*-nitrosoindoline (**17**) in 43% yield. This is anomalous, since the amido carbonyl carbon in the aromatic *N*-nitrosamide (**3**) is positively polarized compared with that of ureido cabonyl in aromatic *N*-nitrosoureas (**1, 2**).[11] Compound **3** should thus favor diazo ester rearrangement (path B in Chart 1) rather than the N–NO bond cleavage (path A). This can be explained as due to interaction between the *N*-nitroso compound and indoline. We reported that aromatic *N*-nitroso compounds can adopt two conformers.[11] One, with a co-planar phenyl–N–NO part, may undergo NO radical fission (path A) and the other, in a twist form, may undergo diazo ester rearrangement (path B). Indoline nitrogen is attracted to the polarized carbonyl carbon of *N*-nitrosamide in a twist conformer (Chart 6). Approach of the nitroso group to the carbonyl and formation of the diazo ester intermediate are inhibited. Consequently, *O*-nitroso intermediate formation by N–NO bond radical cleavage is accelerated.

Aromatic *N*-nitrosamines (**8**) are of particular interest, for they can not form *O*-nitroso compounds corresponding to those produced from *N*-nitrosoureas and *N*-nitrosamides. The transnitrosation of *N*-substituted *N*-nitrosanilines occurred in the order of phenyl > methyl > *tert*-butyl as *N*-substituents. *N*-Nitrosodiphenylamine (**8h**) produced the NO radical in organic solvents[6c,d] and nitrosated piperidine or diethylamine *via* the nitroxide radical intermediate, $Ph_2N\text{-}N(O)\text{-}N<$ obtained by reaction of **8h** and secondary amines.[6d] A similar intermediate may be expected in the present reaction.

The ease of radical cleavage of the N–NO bond would appear to be reflected by the C form in the resonance hybrid, $Ar\text{–}\overset{|}{N}\text{–}N=O$ (A) ↔ $Ar\text{–}\overset{|}{N}^{+}=N\text{–}O^{-}$ (B) ↔ $Ar^{-}=\overset{|}{N}^{+}\text{–}N=O$ (C) (Ar = aromatic ring). A contribution of the resonance hybrid C has not been observed in aliphatic nitrosamines. Perhaps the N–NO bond radical fission of aromatic *N*-nitroso compounds occurs through one-electron transfer with a single bond contribution at the N–NO bond in a transition state.

Since the π electronic system of the aromatic ring and lone pair of nitrogen bonded to nitroso group are conjugated, N–NO bonds in aromatic *N*-nitrosamines easily undergo radical cleavage compared with aliphatic

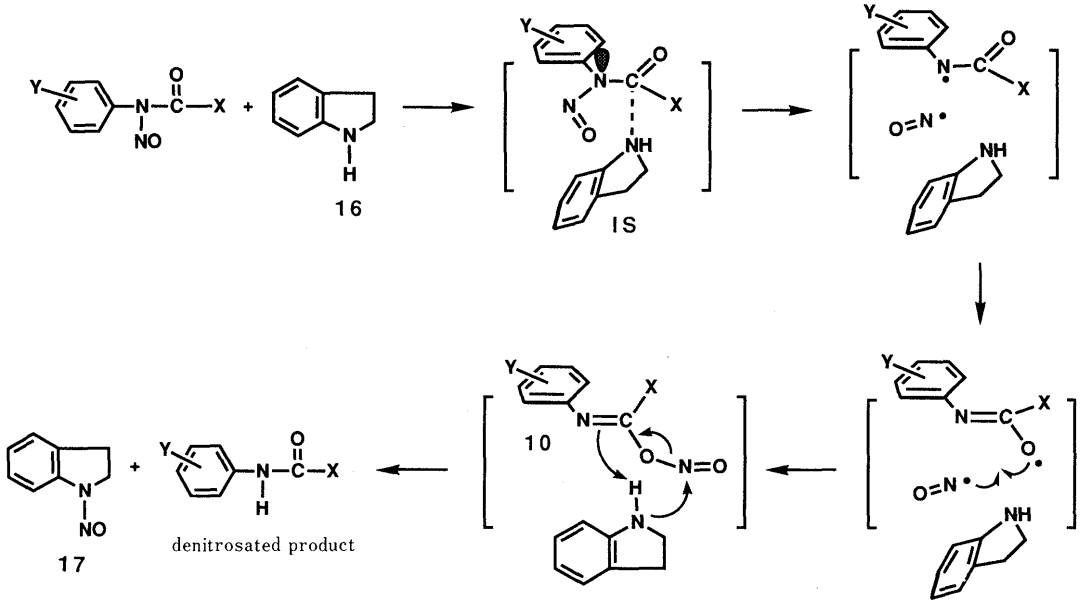

Chart 6

N-nitroso compounds. We previously discussed conjugation based on a ^{13}C-NMR experiment in which the *ortho*-carbons of phenyl in *N*-methyl-*N*-nitroso-4-toluidine (**8a**) resonated at higher field than those of *N*-*tert*-butyl-*N*-nitroso-4-toluidine (**8i**).[11] This may have been due to differences in the degree of twisting about phenyl–N(NO) bonds in the nitrosamines **8**. Conjugation in compound **8i** having a bulky *tert*-butyl group is weakened compared with that in other nitrosamines (**8a, h**)[11] having a methyl or phenyl group owing to twisting of the Ph–N(NO) bond for steric reasons. The N–NO bond of **8i** is thus thermally stable.

The N–NO bonds of trisubstituted *N*-aryl-*N*-nitrosoureas (**1a—g**) are likely to be disrupted by an additional effect, besides conjugation, *i.e.*, the steric hindrance of the N^3 substituents and ureido carbonyl oxygen directed towards the nitroso oxygen.[11] Consequently, the 2-tolyl derivatives (**1e, f**) having non-conjugated N–NO bonds due to twisting caused by steric hindrance between *ortho* methyl and nitroso groups is less transnitrosated than the 4-tolyl derivatives (**1b, c**) having planar forms.[14] For disubstituted *N*-aryl-*N*-nitrosoureas (**2**), transnitrosation is similar to the case of *N*-aryl-*N*-nitrosanilines (**8**) since the effects of steric hindrance due to the N^3-alkyl group and electrostatic repulsion between nitroso oxygen and carbonyl oxygen are small.

Conclusion

1) The important factors for N–NO bond radical cleavage are planar conformation due to resonance and steric effects about the nitroso group in aromatic *N*-nitroso compounds. The ease of transnitrosation reflects the ease of liberation of the NO radical. Because of these effects, aromatic trisubstituted *N*-nitrosoureas (**1**) are easily transnitrosated under mild conditions compared with aromatic disubstituted *N*-nitrosoureas (**2**) and aromatic *N*-nitrosamines (**8**). Aliphatic *N*-nitroso compounds that lack a resonance effect are stable under the present experimental conditions.

2) The basicity of the nitroso acceptor is important. An appropriate decrease in basicity is ideal for non-acidic transnitrosation, since the radical reaction may proceed efficiently under aprotic conditions.

3) In the transnitrosation of acyl-type aromatic *N*-nitroso compounds, the *O*-nitroso intermediate is formed after N–NO bond radical cleavage and acts as a nitrosating agent. That is to say, *O*-nitrosoisourea is the NO-carrying intermediate in the transnitrosation of aromatic *N*-nitrosoureas and *N*-nitrosamides.

NO has been shown to be essential for physiological responses such as vasorelaxation, neurotransmission, and inhibition of tumor cells by cytotoxic macrophages.[15] We examined the cytotoxic effects toward tumor cells of these transnitrosating compounds (**1—4, 8**) which generate the NO radical. These results will be reported later.

Experimental

All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO A-102 spectrometer. Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C-NMR) spectra were taken on a Varian Gemini-300 (300 Mz) NMR spectrometer with tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. High-performance liquid chromatography (HPLC) was done on a JASCO 880-PU chromatograph with an ultraviolet (UV) detector (JASCO 875-UV) operating at 254 nm, using a TSK-Gel LS 310K column (4×300 mm i.d., Toyo Soda) with *n*-hexane and ethyl acetate (80:20 or 87:13, v/v) as a mobile phase. Peak areas were determined with a Hitachi D-2500 Chromato-Integrator. The internal standard employed was 4-methyl-2-nitroacetanilide.[8,9]

Materials *N*-Methylanilines (**9b, e**), *N,N*-dimethylanilines (**11c, e, f**), and indoline (**16**) were purchased from Wako Pure Chemical Industries, Ltd. Aromatic *N*-nitrosoureas (**1, 2**)[7-10] and aliphatic *N*-nitrosoureas (**4, 7**)[9-11] were prepared by nitrosation of the corresponding ureas (**5, 6**) according to the method described in our previous paper. A new aromatic *N*-nitrosourea (**2e**) was prepared by the procedures cited.[16] White-yellow crystals, mp 85—86 °C (dec.). Yield, 30%. *Anal.* Calcd for $C_8H_9N_3O_2$: C, 53.62; H, 5.06; N, 23.45, Found: C, 53.63; H, 5.03; N, 23.45. IR ($CHCl_3$): 3520, 3410 (NH), 1730 (CO) cm^{-1}. ^{13}C-NMR [$CDCl_3$–

CD_3OD (1 : 1), −5—−10 °C] δ: 128.9 (C-1), 127.6 (C-2,6), 130.2 (C-3,5), 140.6 (C-4), 155.5 (CO), 21.4 (Me).

Aromatic *N*-nitrosamides (**3**)[12a] and *N*-nitrosamines (**8**)[11,13] were prepared by the procedures cited.

Preparations of *N*-Methyl- (9a, b, d) and *N*,*N*-Dimethylanilines (11a, b, d)[17] Typical Examples (**9a** and **11a**): A solution of Me_2SO_4 (6.6 g, 0.052 mol) in $CHCl_3$ (100 ml) was added to a solution of 4-toluidine (5.3 g, 0.044 mol) in $CHCl_3$ (100 ml), while the temperature was kept below 5 °C. The reaction mixture was stirred for 3 h, allowed to stand overnight at room temperature, and then evaporated to dryness under reduced pressure. The residue was extracted with $CHCl_3$, and the solvent was evaporated off. The residue was chromatographed on a column of silica gel with *n*-hexane–ether (9 : 1). The first fraction gave *N*,*N*-dimethyl-4-toluidine (**11a**) in 6% yield as an oily product [lit.[17b] bp_{760} 204—206 °C], and the next fraction gave *N*-methyl-4-toluidine (**9a**) in 15% yield as an oily product [lit.[17c] bp_{715} 207—209 °C]. **9a**: ^{13}C-NMR ($CDCl_3$) δ: 147.7 (C-1), 113.0 (C-2, 6), 130.1 (C-3, 5), 126.6 (C-4), 31.0 (N-Me), 20.4 (Me). **11a**: ^{13}C-NMR ($CDCl_3$) δ: 149.4 (C-1), 113.6 (C-2, 6), 130.0 (C-3, 5), 126.4 (C-4), 41.1 (N-Me), 20.3 (Me).

Other *N*-alkylanilines (**9b, d**) and *N*,*N*-dialkylanilines (**11b, d**) were similarly prepared. *N*-Methyl-4-methoxyaniline (**9b**): oily product [lit.[17d] bp_{19} 135—136 °C]. Yield, 66.5%. ^{13}C-NMR ($CDCl_3$) δ: 144.2 (C-1), 115.3 (C-2, 6), 114.1 (C-3, 5), 152.6 (C-4), 31.7 (N-Me), 56.0 (Me). *N*-Methyl-4-chloraniline (**9d**): oily product [lit.[17e] bp_{764} 239—240 °C]. Yield, 15.5%. ^{13}C-NMR ($CDCl_3$) δ: 140.8 (C-1), 120.0 (C-2, 6), 129.4 (C-3, 5), 121.9 (C-4), 30.8 (N-Me). *N*,*N*-Dimethyl-4-methoxyaniline (**11b**): mp 48.0—48.5 °C [lit.[17b] mp 37—38.5 °C]. Yield, 14.5%. ^{13}C-NMR ($CDCl_3$) δ: 42.0 (N-Me). 56.0 (Me). *N*,*N*-Dimethyl-4-chloraniline (**11d**): mp 31.5—32.5 °C [lit.[17b] mp 32—32.5 °C]. Yield, 4.0%. ^{13}C-NMR ($CDCl_3$) δ: 148.5 (C-1), 113.8 (C-2, 6), 129.4 (C-3, 5), 121.9 (C-4), 30.8 (N-Me).

Preparations of *N*-Nitrosanilines (8) and *N*-Nitrosoindoline (17) Typical Example (**8e**): A solution of sodium nitrite (170 mg, 2.5 mmol) in water (10 ml) was added in small portions to a solution of *N*-methyl-4-nitraniline (**9e**) (305 mg, 2.0 mmol) in 20% HCl (15 ml) under ice-water cooling. The reaction mixture was stirred for 30 min below 0 °C, then cold water (20 ml) was added and the whole was neutralized with sodium bicarbonate, then extracted with CH_2Cl_2. The CH_2Cl_2 layer was filtered through a silicone-treated filter paper (1 ps phase separators, Whatman Ltd.) and evaporated under reduced pressure. The residue was recrystallized from ether to give *N*-methyl-*N*-nitroso-4-nitraniline (**8e**), mp 98.5—100 °C (dec.) [lit.[18a] mp 100 °C]. Yield, 306 mg (84%). ^{13}C-NMR (CDCl3) δ: 147.6 (C-1), 118.3 (C-2, 6), 125.7 (C-3, 5), 146.5 (C-4), 30.2 (N-Me).

Other nitrosamines (**8a, b, d, g, 17**) were similarly prepared. Compound **8b** was purified by silica gel column chromatography with a mixture of *n*-hexane and ether. *N*-Methyl-*N*-nitroso-4-toluidine (**8a**): mp 47.5—49.5 °C [lit.[18b] mp 52.4 °C]. Yield, 96.5%. ^{13}C-NMR ($CDCl_3$) δ: 140.0 (C-1), 119.3 (C-2,6), 129.8 (C-3, 5), 137.2 (C-4), 31.4 (N-Me), 20.8 (Me). *N*-Methyl-*N*-nitroso-4-methoxyaniline (**8b**): oily product [lit.[18c] mp 48—49 °C]. ^{13}C-NMR ($CDCl_3$) δ: 136.2 (C-1), 121.5 (C-2, 6), 115.0 (C-3, 5), 159.5 (C-4), 32.3 (N-Me), 55.7 (*N*-Me). *N*-Methyl-*N*-nitroso-4-chloraniline (**8d**): mp 48—48.5 °C [lit.[18d] mp 51 °C]. Yield, 80.8%. ^{13}C-NMR ($CDCl_3$) δ: 140.8 (C-1), 120.0 (C-2, 6), 129.4 (C-3, 5), 132.7 (C-4), 30.8 (N-Me). *N*-Benzyl-*N*-nitrosaniline (**8g**): mp 57.5—58.5 °C [lit.[18e] mp 57—58 °C]. Yield, 75.4%. *N*-Nitrosoindoline (**17**): mp 83—84 °C [lit.[18f] mp 83—84 °C]. Yield, 95.5%.

Transnitrosation of *N*-Nitrosourea (1a) to *N*-Alkylanilines (9) 3,3-Dibenzyl-1-(4-tolyl)-1-nitrosourea (**1a**) (7.3 mg, 0.02 mmol) was added to a solution of an *N*-alkylaniline (**9**) (0.02 mmol) in CCl_4 (l0 ml). The solution was kept at 20 °C or 33 °C. The yields of the corresponding *N*-nitrosaniline (**8**), denitrosated 3,3-dibenzyl-1-(4-tolyl)urea (**5a**) and 3,3-dibenzyl-1-(2-nitro-4-tolyl)urea (**14a**) were determined by the HPLC method. The results are shown in Table I and Fig. 2.

Formation of *N*-Alkyl-*N*-nitrosanilines (8) from *N*,*N*-Dimethylanilines (11) by Demethylating Transnitrosation Compound **1a** (7.3 mg, 0.02 mmol) was added to a solution of an *N*,*N*-dimethylaniline (**11**) (0.02 mmol) in CCl_4 (10 ml). The solution was kept at 20 °C or 33 °C. The yields of *N*-methyl-*N*-nitrosanilines (**8**) were determined by the HPLC method, and the results are shown in Table III.

When **1a** was allowed to react with **11a—d** in CCl_4 for 4.5 h at 20 °C, *N*,*N*-dimethyl-2-nitranilines were produced in 8.9—16.6% yields, besides the formation of *N*-methyl-*N*-nitrosanilines (**8**) (Table III). These nitro compounds were identified by comparison with authentic samples.[19a–c] *N*,*N*-Dimethyl-2-nitro-4-toluidine: oily product. Yield, 16.6%. ^{1}H-NMR ($CDCl_3$)[19a,b] δ: 2.30 (3H, s), 2.85 (6H, s), 6.96 (1H, d, J=8.4 Hz), 7.23 (1H, dd, J=8.1 Hz), 7.58 (1H, d, J=1.44 Hz). ^{13}C-NMR ($CDCl_3$) δ: 144.9 (C-1), 140.3 (C-2), 134.7 (C-3), 130.5 (C-4), 126.8 (C-5), 118.9 (C-6), 42.9 (N-Me), 20.1 (Me). *N*,*N*-Dimethyl-2-nitro-4-methoxylaniline: oily product. Yield, 15.4%. ^{1}H-NMR[19a,b] ($CDCl_3$) δ: 2.80 (3H, s), 3.79 (6H, s), 7.0—7.3 (3H, m). ^{13}C-NMR ($CDCl_3$) δ: 141.5 (C-1), 141.8 (C-2), 110.0 (C-3), 153.4 (C-4), 121.3 (d, C-5, 6), 43.7 (N-Me), 56.1 (MeO). *N*,*N*-Dimethyl-2-nitro-4-chloraniline: mp 55—56 °C [lit.[19c] mp 55—56 °C]. Yield, 8.9%. ^{1}H-NMR ($CDCl_3$) δ: 2.89 (6H, s), 6.97 (1H, d, J=8.1 Hz), 7.35 (1H, dd, J=9.0 Hz), 7.77 (1H, d, J=2.7 Hz). ^{13}C-NMR ($CDCl_3$) δ: 145.4 (C-1), 139.3 (C-2), 133.7 (C-3), 122.8 (C-4), 126.7 (C-5), 119.7 (C-6), 42.6 (N-Me).

Demethylating nitrosation using other *N*,*N*-dialkylanilines was also undertaken similarly. The results are shown in Table III.

Isolation of *N*-Methyl-*N*-nitrosanilines (**8**) after Demethylating Transnitrosation: Compound **1a** (180 mg, 0.5 mmol) was added to *N*,*N*-dimethylaniline (**11c**) (60 mg, 0.5 mmol) in CCl_4 (10 ml). The solution was allowed to stand overnight at room temperature. The solvent was evaporated off, and the residue was chromatographed on a column of silica gel with a mixture of *n*-hexane and ether (4 : 1). The first fraction gave 3,3-dibenzyl-1-(4-tolyl)triazene (**15a**) (1 mg). The second fraction gave **11c** (1.65 mg). The third fraction gave *N*,*N*-dibenzyl-*N*-nitrosamine (3.7 mg). The last fraction gave *N*-methyl-*N*-nitrosaniline (**8c**) (31.8 mg, 47%).

Detection of Formaldehyde Formed in Demethylating Transnitrosation: When the reagent for formaldehyde detection (Tokyo Kasei Ltd.) was added to the reaction mixture of **1a** and **11c** dissolved in CCl_4, the solution changed from yellow to red color as the reaction progressed.

Transnitrosation of *N*-Nitrosoureas (1a—c) to the Urea (6b) A 3,3-dialkyl-1-(4-tolyl)-1-nitrosourea (**1a—c**) (0.034 mmol) was added to a suspension of 3-methyl-1-(4-tolyl)urea (6b) (5.74 mg, 0.034 mmol) in CCl_4 (10 ml) containing an internal standard. The solution was kept at 33 °C for 6 h. The time course of the yields of 3-methyl-1-(4-tolyl)-1-nitrosourea (**2b**) and 3-methyl-1-(4-tolyl)-3-nitrosourea (**4b**), which were determined by the HPLC method, was plotted.

In the reaction of 3,3-diethyl-1-(4-tolyl)-1-nitrosourea (**1c**) with **6b**, the denitrosation product 3,3-diethyl-1-(4-tolyl)urea (**5c**), 3,3-diethyl-1-(2-nitro-4-tolyl)urea (**14c**), and 3,3-diethyl-1-(4-tolyl)triazene (**15c**) were also determined by the HPLC method. These products (**2b, 4b, 5c, 14c, 15c**) were identified by comparison with authentic samples obtained by the method described in our previous paper.[9,10] The yields of products **2b, 4b, 5c, 14c** and **15c** were 8%, 16%, 24%, 47% and 12%, respectively. The results are shown in Table IV and Fig. 3a,b.

Transnitrosation of Various *N*-Nitrosoureas (1—4, 7, 8) to Indoline (16) Typical Example (**1a**): Compound **1a** (7.3 mg, 0.02 mmol) was added to a solution of indoline (**16**) (0.02 mmol) in CCl_4 (10 ml). The solution was kept at 20 °C or 33 °C. The yield of *N*-nitroindoline (**17**) was determined by the HPLC method. The transnitrosation to indoline using other *N*-nitroso compounds was examined similarly, and the results are shown in Table V.

Kinetics of the Transnitrosation All rate measurements were carried out at 20 °C using HPLC, by following the disappearance of the peak of **1a** in CCl_4 containing *N*-alkylanilines (**9a—f**) or containing 3-methyl-1-(4-tolyl)urea (**6b**) in the presence of an internal standard as a function of time using a chart recorder. The results are given in Tables II and IV.

References

1) B. C. Challis, M. R. Osborne, *J. Chem. Soc., Perkin Trans. 2*, **1973**, 1526.
2) *a*) S. S. Singer, B. B. Cole, *J. Org. Chem.*, **46**, 3461 (1981); *b*) R. Bonnett, P. Nicolaidou, *J. Chem. Soc., Perkin Trans. 1*, **1979**, 1969.
3) *a*) S. S. Singer, W. Lijinsky, G. M. Singer, *Tetrahedron Lett.*, **1977**, 1613; *b*) S. S. Singer, G. M. Singer, B. B. Cole, *J. Org. Chem.*, **45**, 3931 (1980).
4) S. A. Leach, M. Thompson, M. Hill, *Carcinogenesis*, **8**, 1907 (1987).
5) H. Ohsima, S. Calmels, B. Pignatelli, P. Vincent, H. Bartsch, *IARC Sci. Publ.*, **84**, 384 (1987).
6) *a*) C. L. Bumgardner, K. S. McCallum, J. P. Freeman, *J. Am. Chem. Soc.*, **83**, 4417 (1961); *b*) D. B. Parihar, S. P. Sharma, *Chem. Ind.* (London), **28**, 1227 (1966); *c*) P. Welzel, *Chem. Ber.*, **104**, 808 (1971); *d*) A. J. Buglass, B. C. Challis, M. R. Osborne, *IARC Sci. Publ.*, **9**, 94 (1974).

7) M. Tanno, S. Sueyoshi, S. Kamiya, *Chem. Pharm. Bull.*, **38**, 2644 (1990).
8) S. Sueyoshi, M. Tanno, *Chem. Pharm. Bull.*, **33**, 488 (1985).
9) M. Tanno, S. Sueyoshi, *Chem. Pharm. Bull.*, **35**, 1360 (1987).
10) M. Tanno, S. Sueyoshi, S. Kamiya, *Chem. Pharm. Bull.*, **38**, 49 (1990).
11) M. Tanno, S. Sueyoshi, *Chem. Pharm. Bull.*, **35**, 1353 (1987).
12) *a*) D. H. Hey, J. Stuart-Webb, G. H. Williams, *J. Chem. Soc.*, **1952**, 4657; *b*) P. Miles, H. Suschitzky, *Tetrahedron*, **18**, 1369 (1962).
13) *a*) R. N. Leoppky, W. Tomasik, *J. Org. Chem.*, **48**, 2751 (1983); *b*) G. Verardo, A. G. Giumanini, P. Strazzolini, *Tetrahedron*, **46**, 4303 (1990).
14) K. Yamaguchi, G. Matsumura, M. Tanno, S. Sueyoshi, N. Miyata, *Acta. Crystallogr., Sect. C*, **48**, 1051 (1992).
15) S. Moncada, R. M. J. Palmer, E. A. Higgs, *Pharmacol. Rev.*, **43**, 109 (1991).
16) R. Uehara, *Bull. Chem. Soc. Jpn.*, **33**, 12 (1960).
17) *a*) F. Ullmann, *Justus Liebigs Ann. Chem.*, **327**, 104 (1903); *b*) D. G. Thomas, J. H. Billman, C. E. Davis, *J. Am. Chem. Soc.*, **68**, 895 (1946); *c*) *Beilstein*, **12**, 902; *d*) *Beilstein*, **13**, 442; *e*) *Beilstein*, **12**, 609.
18) *a*) *Beilstein*, **1671**, 727; *b*) C. E. Looney, W. D. Phillips, E. L. Reilly, *J. Am. Chem. Soc.*, **79**, 6136 (1957); *c*) S. F. Nelsen, D. H. Heath, *ibid.*, **91**, 6452 (1969); *d*) *Beilstein*, **12**, 609; *e*) J. W. Pensabene, W. Fiddler, C. J. Dooley, R. C. Doerr, A. E. Wasserman, *J. Agr. Food Chem.*, **20**, 274 (1972); *f*) J. T. D'Agostino, H. H. Jaffé, *J. Am. Chem. Soc.*, **92**, 5160 (1970).
19) *a*) T. Yokoyama, *Aust. J. Chem.*, **27**, 915 (1974); *b*) *Idem*, *ibid.*, **29**, 1469 (1976); *c*) N. Ishikawa, K. Kuroda, *Nippon Kagaku Zasshi*, **88**, 1010 (1967).